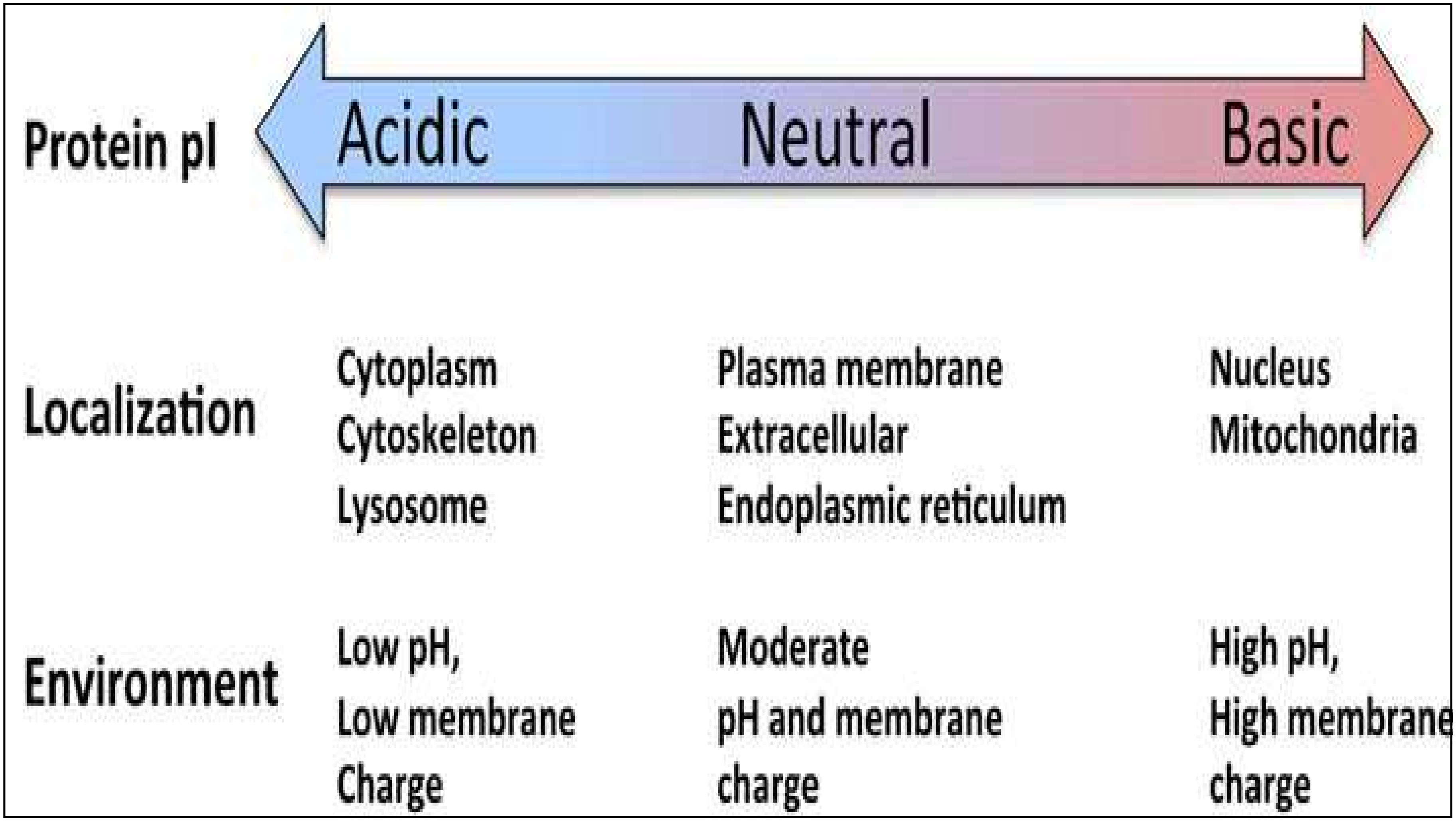
Charge d’une protéine et pH

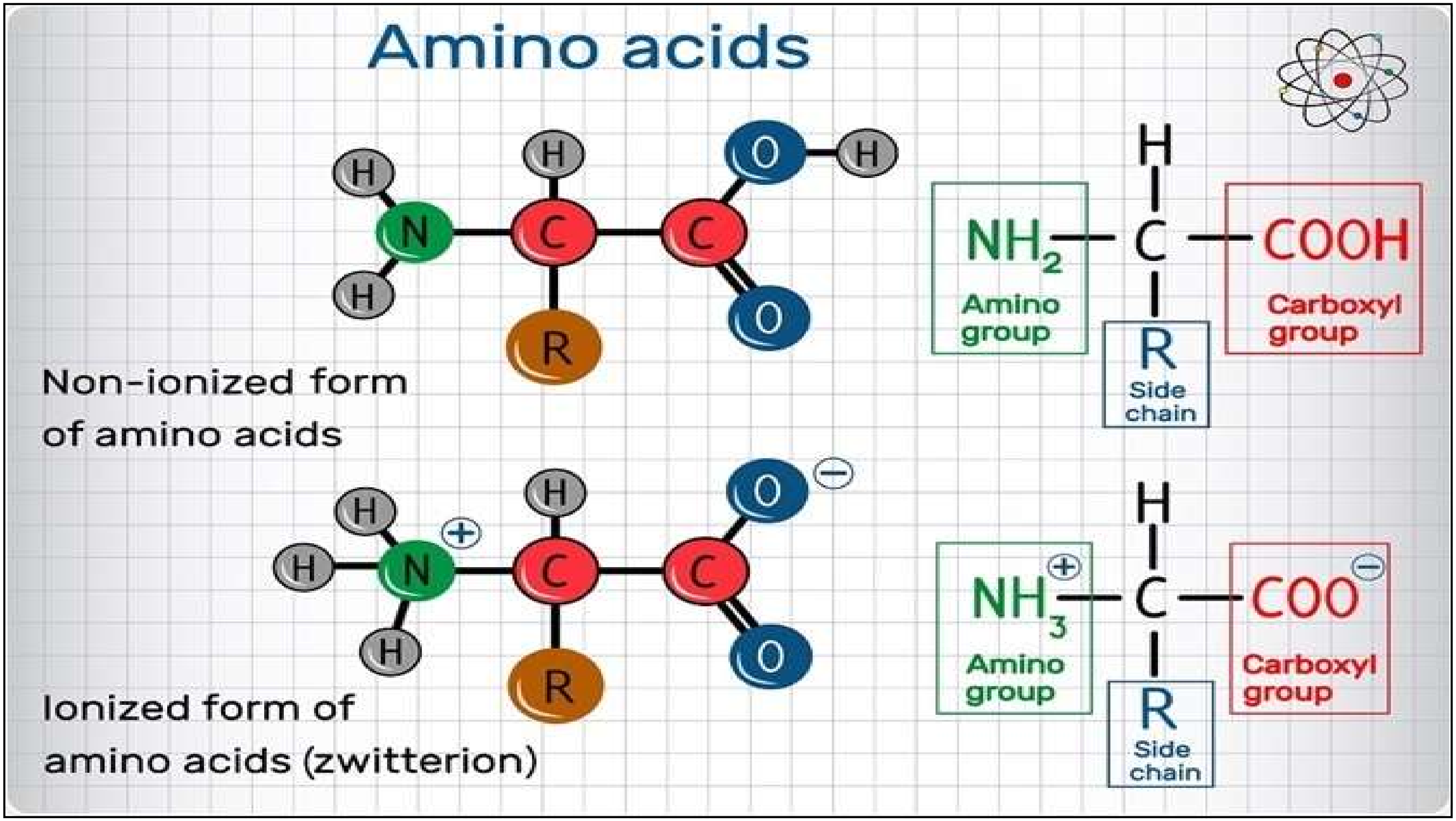
The separation of biomolecules, particularly proteins, in the presence of an electric field (e.g., electrophoresis) has given rise to an array of methodologies to reduce the complexity of samples to probe the physiochemical properties of such biomolecules.

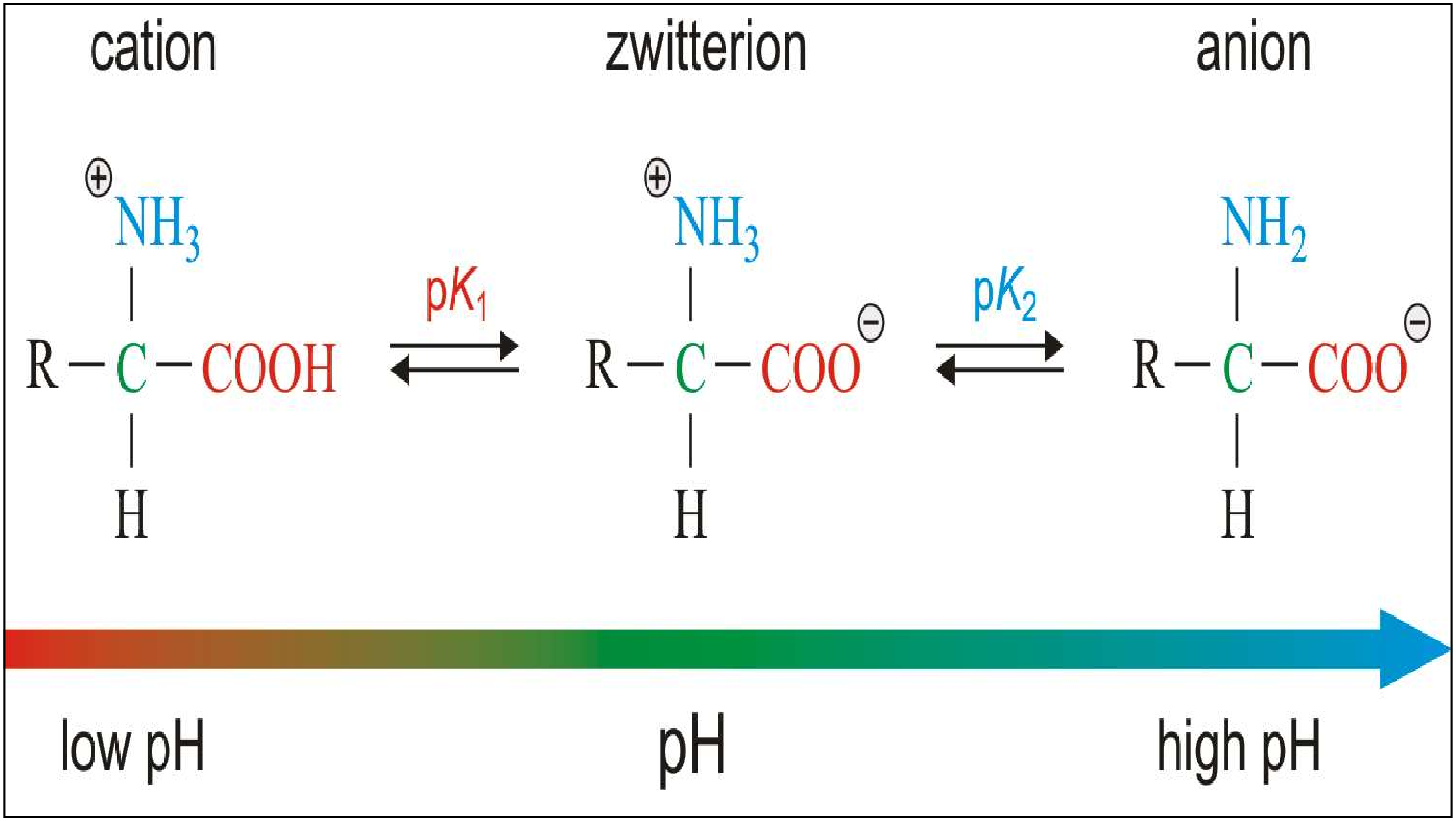
Proteins and peptides represent possibly the most highly studied class of molecules that are interrogated by electrophoretic methods.

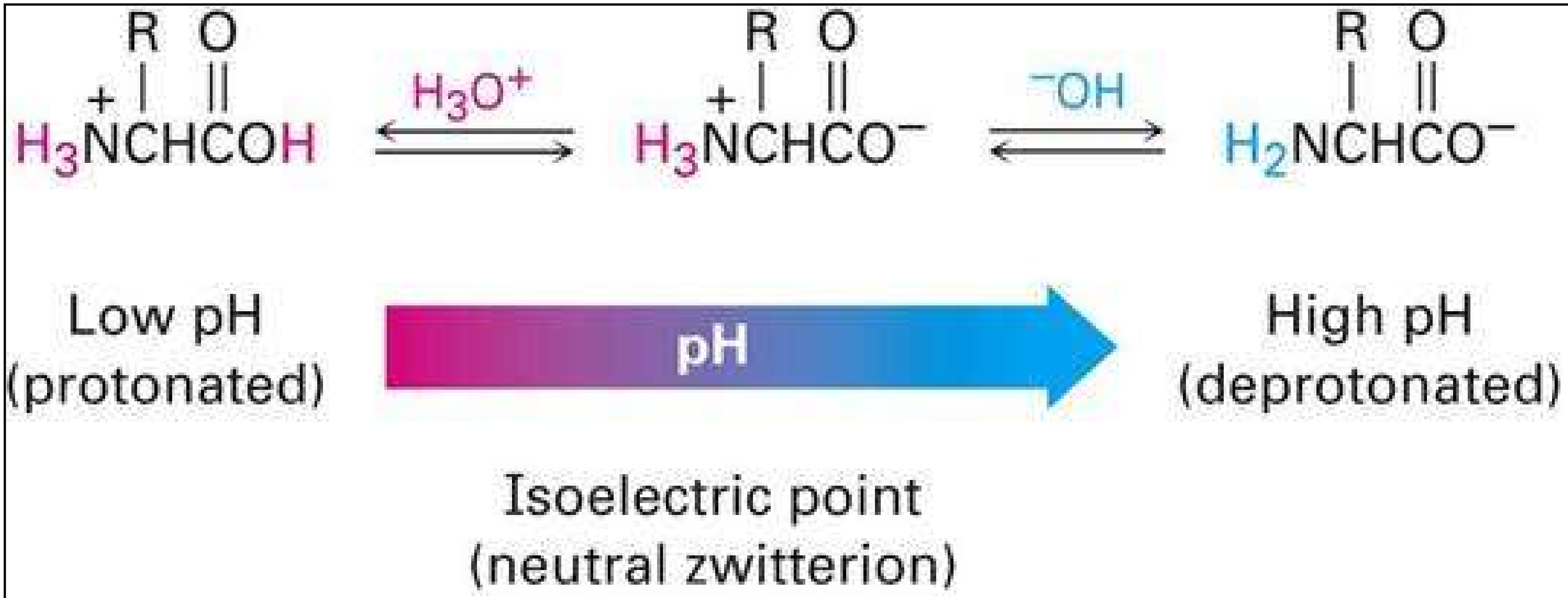
These methods include: agarose and polyacrylamide gel electrophoresis, two-dimensional gel electrophoresis (2DE), capillary electrophoresis, isotachophoresis.



Protein pI	Acidic	Neutral	Basic
Localization	Cytoplasm Cytoskeleton Lysosome	Plasma membrane Extracellular Endoplasmic reticulum	Nucleus Mitochondria
Environment	Low pH, Low membrane Charge	Moderate pH and membrane charge	High pH, High membrane charge


Amino acids
H
H
N
H
C
R
C
O
H
O
NH_2 — C — COOH
H
R
Amino group
Side chain
Carboxyl group
Non-ionized form of amino acids
H
H
H
N ⊕
H
C
R
C
O ⊖
O
NH_3^+ — C — COO^-
H
R
Amino group
Side chain
Carboxyl group
Ionized form of amino acids (zwitterion)

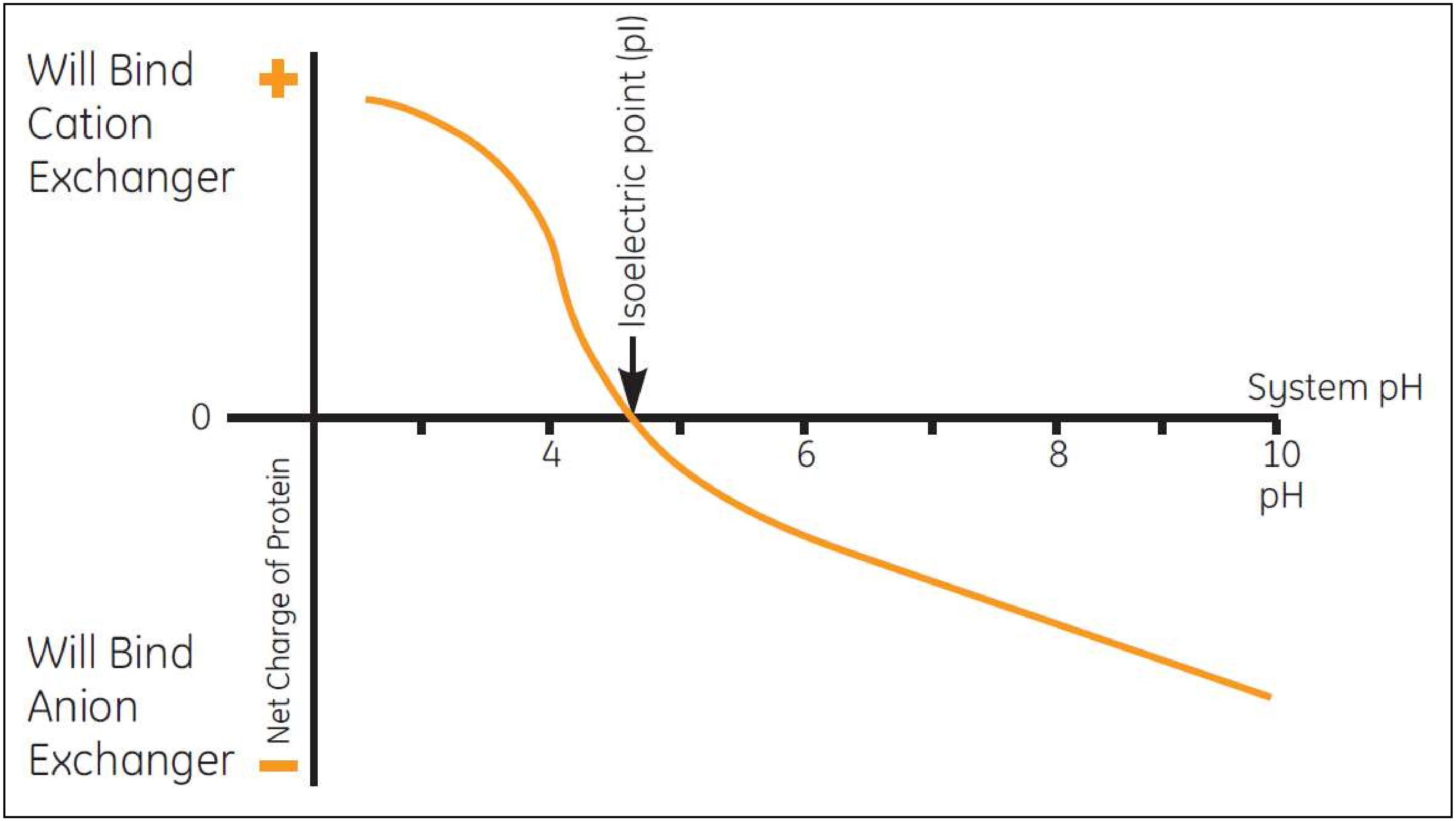

cation
zwitterion
anion
⊕
NH_3
R – C – COOH
pK_1
⊕
NH_3
R – C – $COO^{\ominus}$
pK_2
NH_2
R – C – $COO^{\ominus}$
H
H
H
low pH
pH
high pH



$$H_3\overset{+}{N}\overset{R}{\overset{|}{C}}H\overset{O}{\overset{\|}{C}}OH \overset{H_3O^+}{\rightleftharpoons} H_3\overset{+}{N}\overset{R}{\overset{|}{C}}H\overset{O}{\overset{\|}{C}}O^- \overset{^-OH}{\rightleftharpoons} H_2N\overset{R}{\overset{|}{C}}H\overset{O}{\overset{\|}{C}}O^-$$

Low pH
(protonated)
pH
High pH
(deprotonated)
Isoelectric point
(neutral zwitterion)

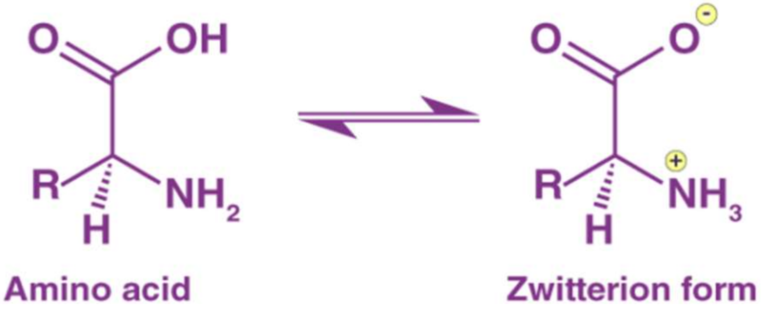
- At its isoelectric pH, a molecule will not migrate in an electric field. This property can be put to use in separation methods.


Will Bind
Cation
Exchanger
+
Isoelectric point (pI)
System pH
0
4
6
8
10
pH
Net Charge of Protein
Will Bind
Anion
Exchanger
−

- **Zwitterions**
- The isoelectric point corresponds to what is known as the zwitterionic form of a protein.
- Zwitterion possesses discrete functional groups, each of which carry a positive or negative charge.
- A zwitterionic amino acid consists of a negative carboxylate ion and a positive ammonium ion alongside a charge on their side chain, which varies between amino acids.

Zwitterions

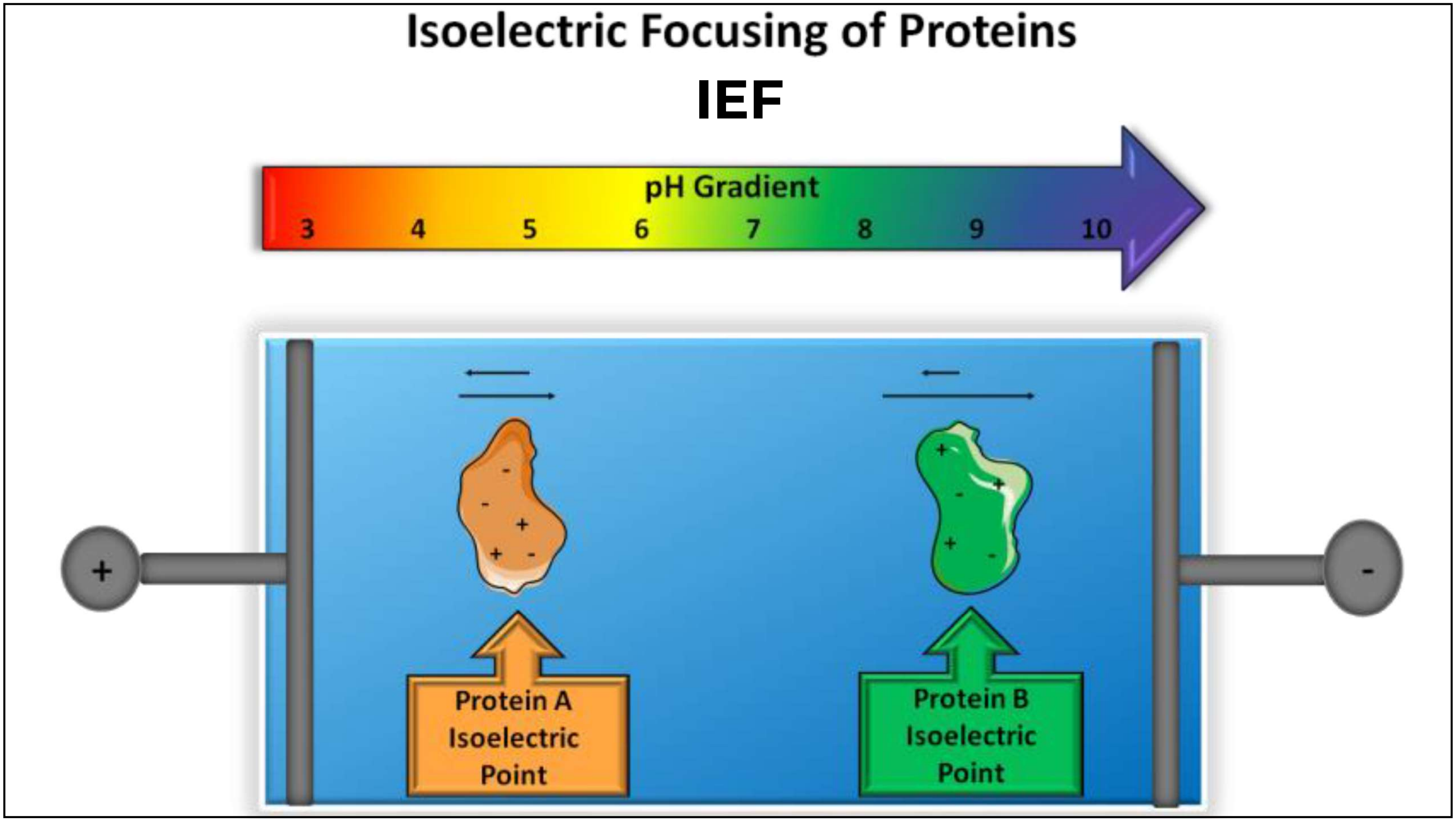
Molecules/ions containing an equal number of both positively and negatively charged functional groups



Charge d’une protéine et pH

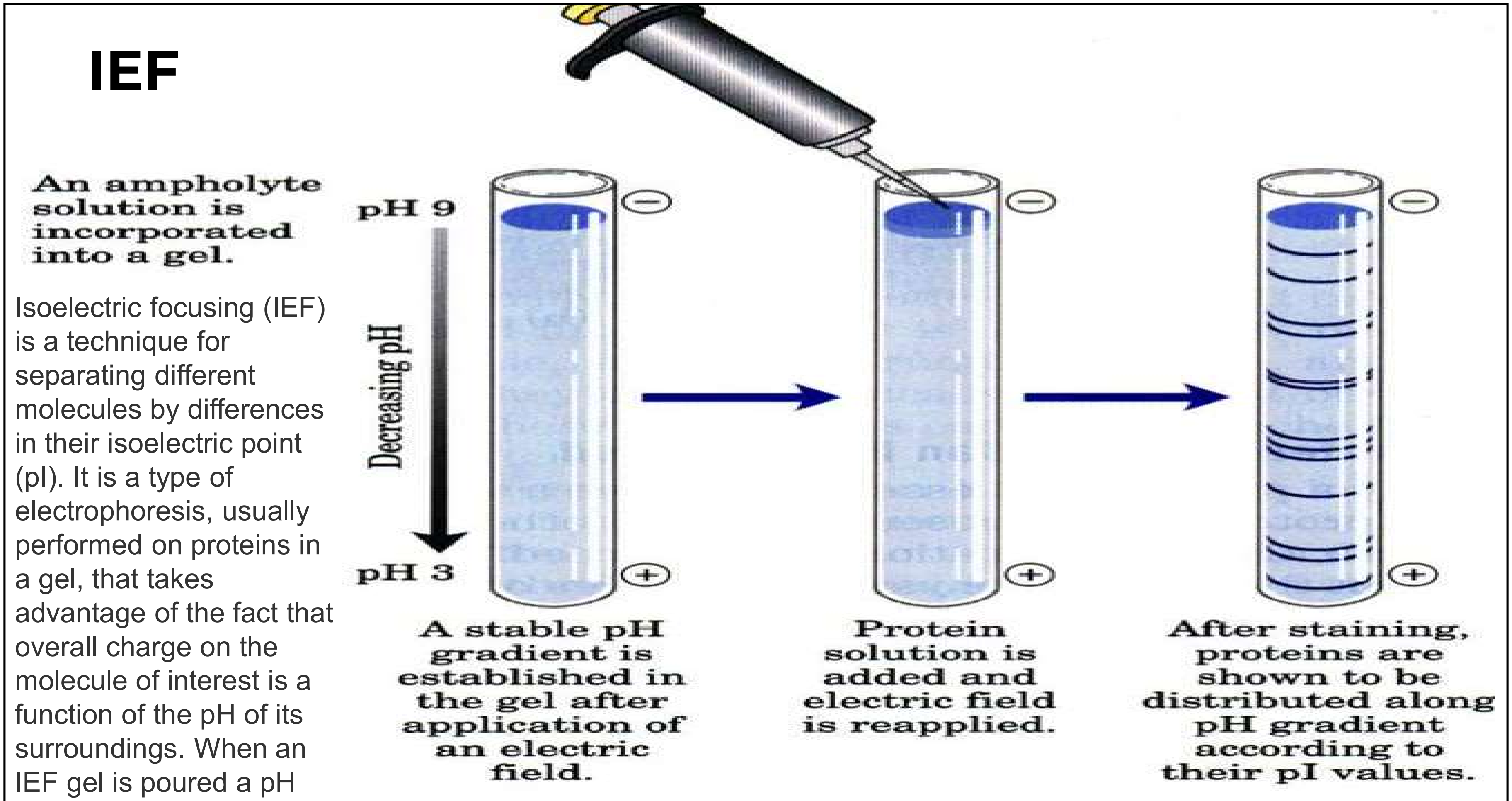
isoelectric point vs isoionic point

- The isoelectric point is defined as the pH at which no net migration takes place in an electric field
- the isoionic point is defined as the pH at which there is no net charge on the molecule.
- In a deionized solution, the isoelectric and isoionic points are for most purposes identical.
- The isoelectric point is the point at which the overall charge of the protein is zero (a neutral charge).
- Separation of proteins at the isoelectric point is called isoelectric focusing. In isoelectric focusing a gradient of pH and an electric potential are applied across the gel, making one end more positive than the other.
- Separation occurs on the basis of the positive or negative groups present on the molecule.
- If they are positively charged, they will be pulled toward the more negative end of the gel and if they are negatively charged they will be pulled to the more positive end of the gel.
- The proteins applied in the first dimension will migrate through the gel and will accumulate at their isoelectric point. At this stage the protein net charge is zero and therefore does not move in an electric field.

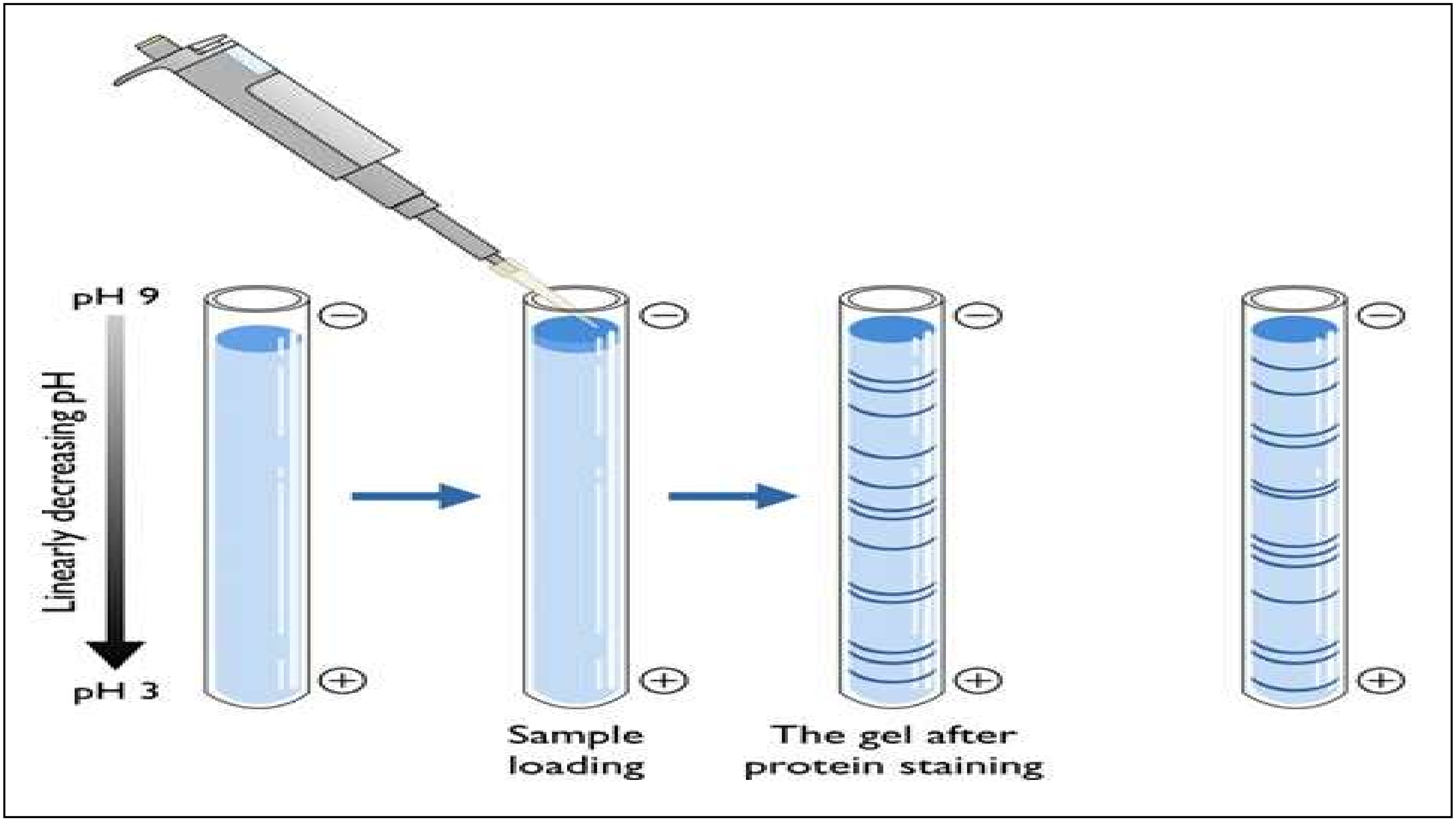

Isoelectric Focusing of Proteins
IEF
pH Gradient
3
4
5
6
7
8
9
10
+
-
Protein A Isoelectric Point
Protein B Isoelectric Point

Charge d’une protéine et pH

IEF


An ampholyte solution is incorporated into a gel.
pH 9
Decreasing pH
pH 3
−
+
A stable pH gradient is established in the gel after application of an electric field.
Protein solution is added and electric field is reapplied.
After staining, proteins are shown to be distributed along pH gradient according to their pI values.

Isoelectric focusing (IEF) is a technique for separating different molecules by differences in their isoelectric point (pI). It is a type of electrophoresis, usually performed on proteins in a gel, that takes advantage of the fact that overall charge on the molecule of interest is a function of the pH of its surroundings. When an IEF gel is poured a pH gradient is established


pH 9
Linearly decreasing pH
pH 3
Sample loading
The gel after protein staining

Capillary isoelectric focusing (cIEF) Technology

- Capillary isoelectric focusing allows the separation of protein/peptide mixtures, protein glycoforms and other charged variants, based on their isoelectric point (pI). A voltage is applied to the anolyte and catholyte tanks, establishing a pH gradient through the capillary.
- Proteins that are applied in the field can be separated based on their net charge, and become immobilized when they reach a pH, which equals to their pI.
- Compared to conventional isoelectric focusing (IEF) in gels, cIEF allows for higher resolution, faster sample analysis with 10 fold lower limit of detection.

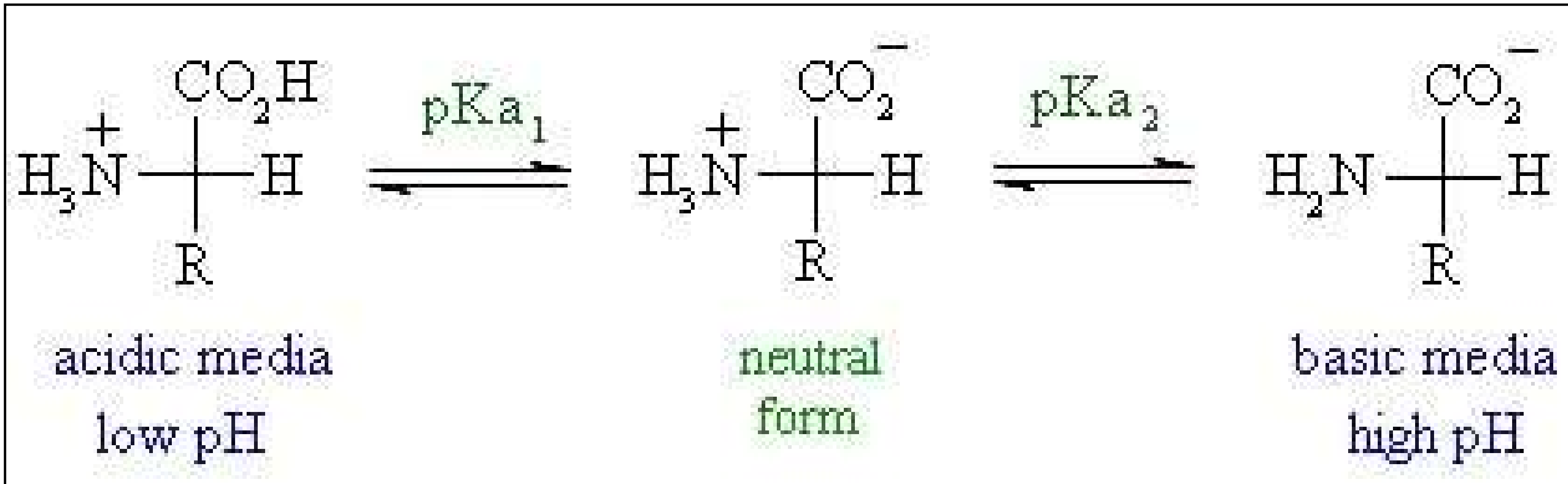
- isoelectric focusing (IEF) provides separation of ampholytic components, molecules that act as weak acids and bases, according to their isoelectric points.
- In IEF, ampholytes travel according to their charge under the influence of an electric field, in the presence of a pH gradient, until the net charge of the molecule is zero (e.g., isoelectric point, pI).
- When considering peptides and proteins, the separation is deemed according to the composition of amino acids and exposed charged residues, which behave as weak acids and bases.
- The migration of the ampholytic species will follow basic principles of electrophoresis; however, the mobility will change in the presence of the pH gradient by slowing down migration at values close to the pI value.

What is the relationship between the pH and pKa of an amino acid?

- The pKa for an amino acid is an acid dissociation constant that refers to the equilibrium between the protonated and deprotonated forms of the backbone amino group, backbone carboxyl group, and any potential acid/base component of the variable group. The net charge of the protein is determined by summing the charge of individual amino acids across the protein.

- pKa relates to the equilibrium constant and defines the transition between two structural forms of an amino acid–protonated and deprotonated. Amino acids possess distinct pKas for the carbonyl group, amino group, and any functional groups on the side chain that may be protonated or deprotonated.

- There is always one more structure than the number of pKa values for amino acids. For example, if there were two pKa values, three structures can be discerned. This is illustrated below using glycine, which has two pKa values – pKa 1 represents the (de)protonation of the carboxyl group and pKa2 represents the (de)protonation of the amine group:


$H_3\overset{+}{N}$
CO_2H
H
R
pKa 1
CO_2^-
pKa 2
H_2N
acidic media
low pH
neutral
form
basic media
high pH

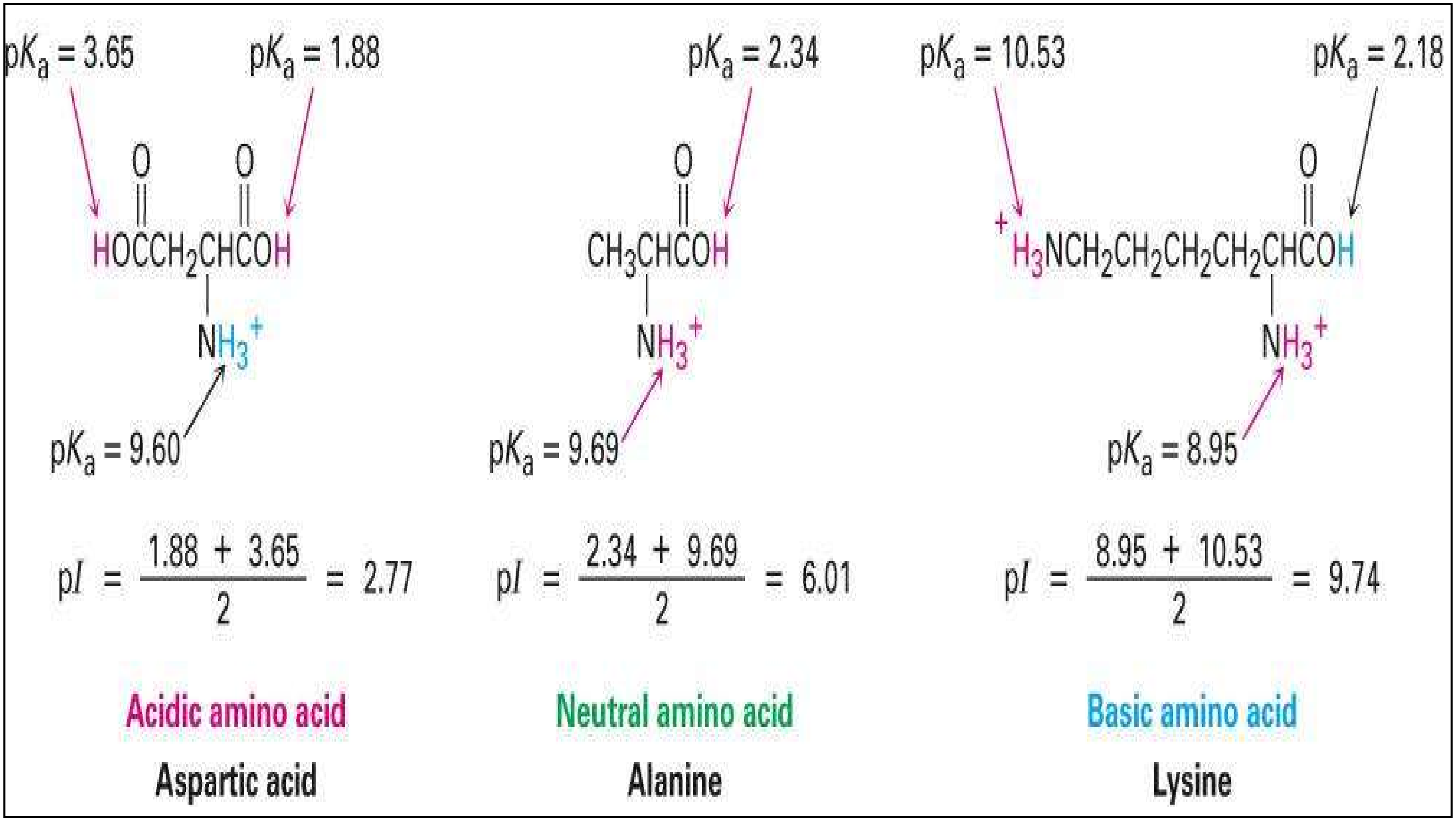
- When the $pH < pK_a$ protonation of the amino and carboxyl groups occurs, resulting in a net +1 positive charge for glycine. When the pH is increased to a value between the two pK_as($pK_a2 > pH > pK_a1$), the carboxyl group is deprotonated while the amino group remains protonated. This represents the zwitterionic form of the amino acid as it simultaneously possesses a positive and negative charge.
- When the $pH > pK_a2$, the amino group is deprotonated and loses its charge. The amino acid now carries a net negative charge of -1. The zwitterionic form of the amino acid can exist at any pH value between pK_a1 and pK_a2.

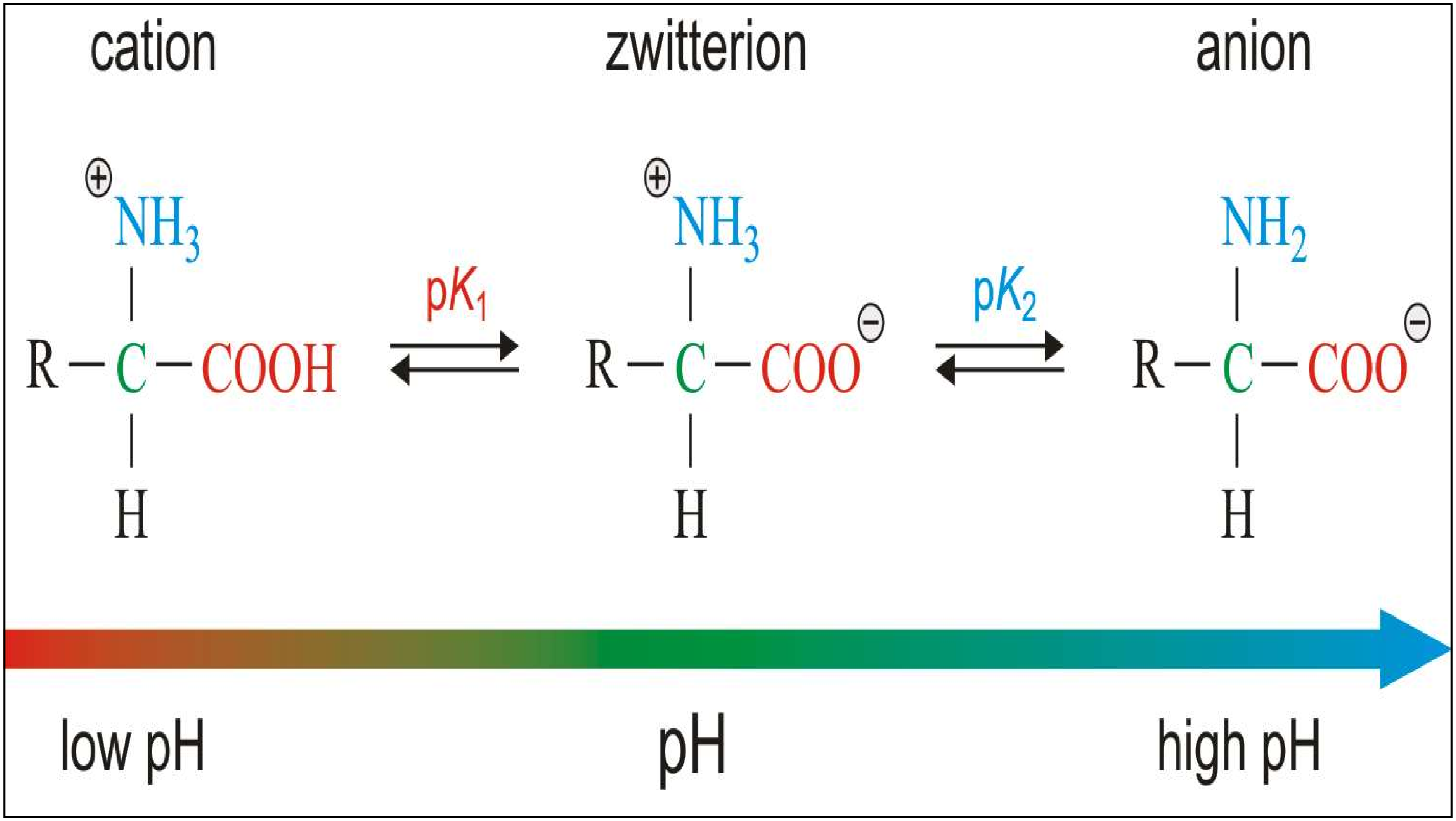
- The pI of any amino acid is the average of the two acid-dissociation constants that involve the neutral zwitterion.
  The 4 amino acids with either a strongly or weakly acidic chain, pI is the average of the two lowest pKa values
- The 13 amino acids with a neutral side chain, pI is the average of pKa1 and pKa2
- The 3 amino acids with a basic side chain, pI is the average of the two highest pKa values

The pI of an amino acid can be calculated by the following equation:

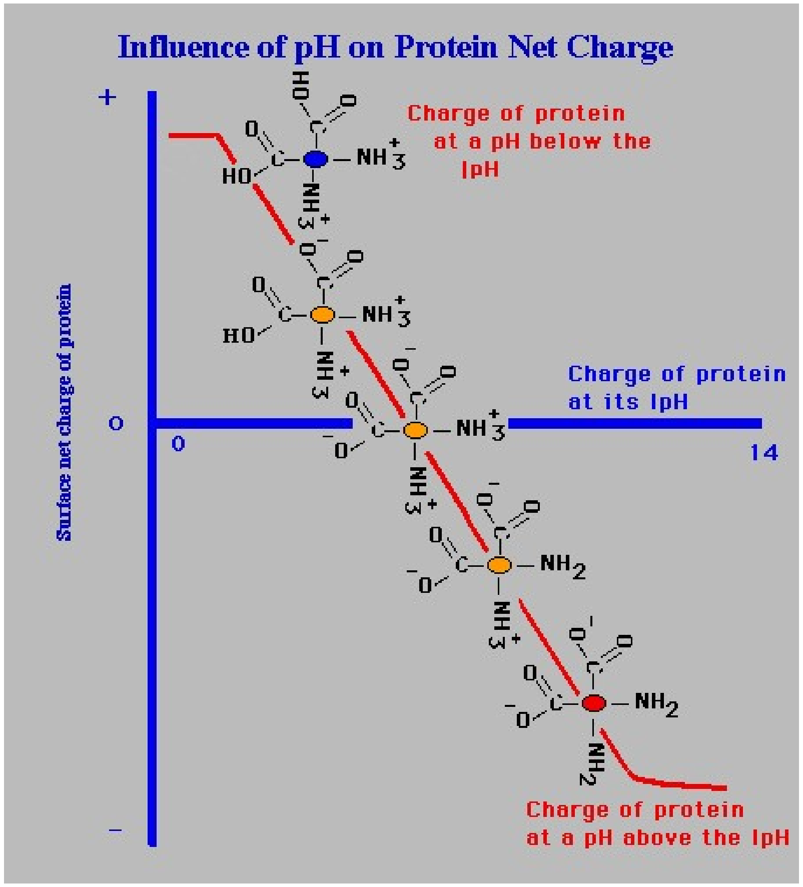
$$\mathrm{pI} = \frac{\mathrm{p}K_{\mathrm{a}1} + \mathrm{p}K_{\mathrm{a}2}}{2}$$

- The fact that amino acids, peptides, and proteins have different pKa values gives rise to the possibility that they can have different charges at a given pH.
- Alanine and histidine, for example, both have net charges of –1 at high pH, above 10; the only charged group is the carboxylate anion. At lower pH, around 5, alanine is a zwitterion with no net charge, but histidine has a net charge of 1 at this pH because the imidazole group is protonated.
- This property is useful in **electrophoresis**, a common method for separating molecules in an electric field.
- This method is extremely useful in determining the important properties of proteins and nucleic acids. The pH at which a molecule has no net charge is called the isoelectric pH, or **isoelectric point** (given the symbol pI).
- At its isoelectric pH, a molecule will not migrate in an electric field. This property can be put to use in separation methods. The pI of an amino acid can be calculated by the following equation:

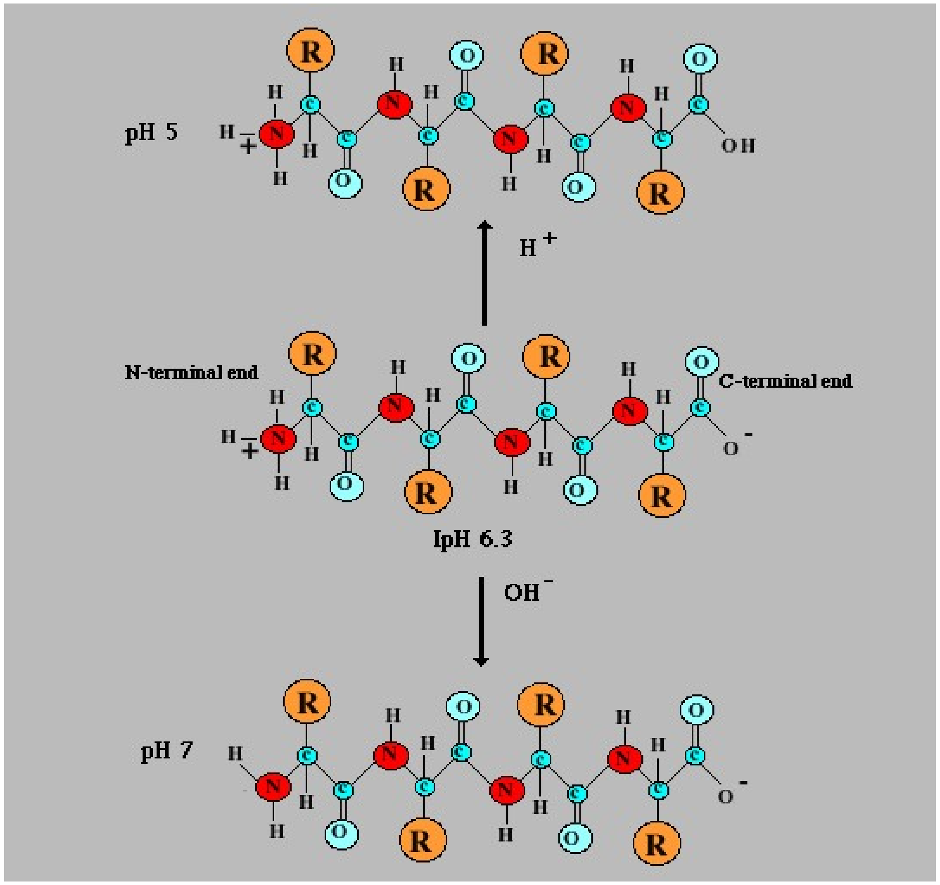

$pK_a = 3.65$
$pK_a = 1.88$
$pK_a = 9.60$
$pI = \frac{1.88 + 3.65}{2} = 2.77$
Acidic amino acid
Aspartic acid
$pK_a = 2.34$
$pK_a = 9.69$
$pI = \frac{2.34 + 9.69}{2} = 6.01$
Neutral amino acid
Alanine
$pK_a = 10.53$
$pK_a = 2.18$
$pK_a = 8.95$
$pI = \frac{8.95 + 10.53}{2} = 9.74$
Basic amino acid
Lysine


cation
zwitterion
anion
$R-C(H)(NH_3^+)-COOH$
pK_1
$R-C(H)(NH_3^+)-COO^-$
pK_2
$R-C(H)(NH_2)-COO^-$
low pH
pH
high pH

Charge d’une protéine et pH


Influence of pH on Protein Net Charge
+
Surface net charge of protein
0
–
0
14
Charge of protein at a pH below the IpH
Charge of protein at its IpH
Charge of protein at a pH above the IpH

Les protéines sont des polymères chargés


pH 5
H⁺
N-terminal end
C-terminal end
IpH 6.3
OH⁻
pH 7
R
N
C
O
H
OH

Protéines et pHi

- Pepsine A (enzyme protéolytique: protéase de l'estomac),

pHi < 1

- Hémoglobine, protéine de transport O_2 (hématies),

pHi ≈ 6,8 - 7

- Trypsine, chymotrypsine, protéases secrétées par le pancréas,

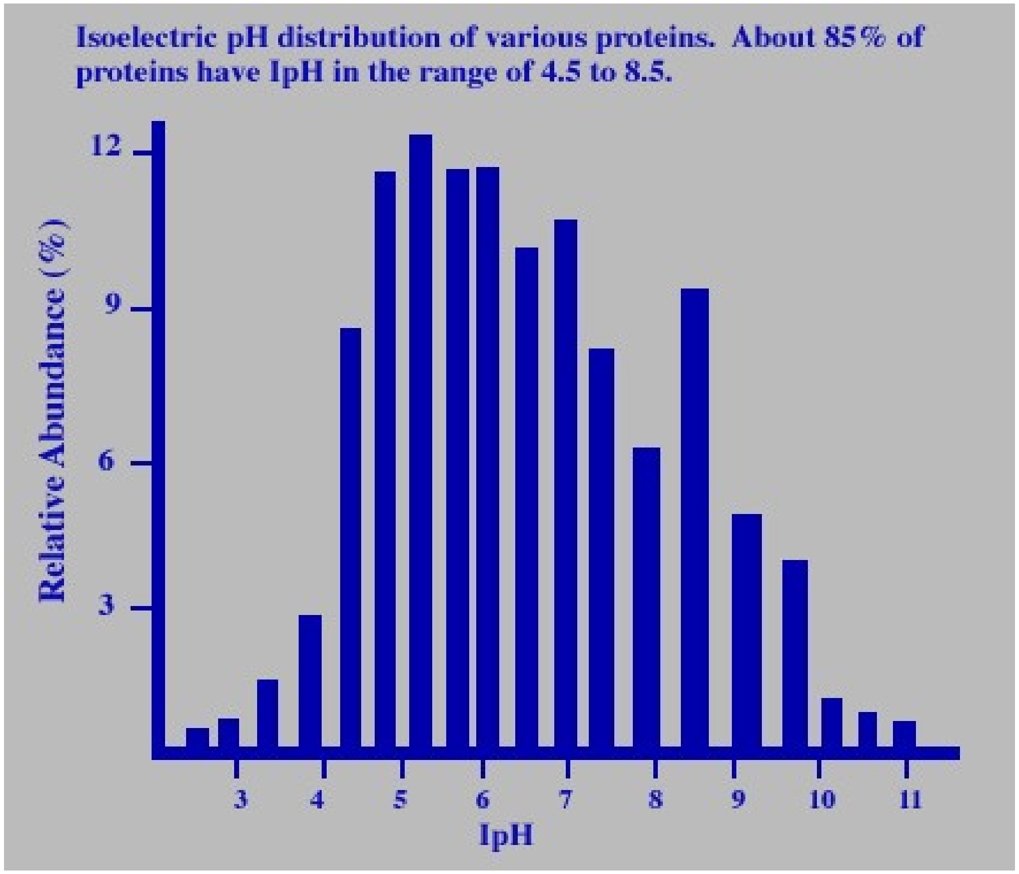
pHi ≈ 9

- Histones, protéines associées à l'ADN dans le chromosome eucaryote, **pHi ≈ 10-11**

- Protamines (polypeptides du sperme des poissons)

pHi ≈ 12

Point isoélectrique des protéines



Isoelectric pH distribution of various proteins. About 85% of proteins have IpH in the range of 4.5 to 8.5.